



First report on pollinating behavior of the small carpenter bee *Ceratina ridleyi* Cockerell (Hymenoptera, Apidae) in *Globba leucantha* var. *bicolor* Holttum (Zingiberaceae)

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Abstract

Small carpenter bees of the genus *Ceratina* are widespread, but little-studied as pollinators despite their presence in various pollinator assemblages. This study documents the importance of *Ceratina ridleyi* as a pollinator of the small-flowered Malaysian ginger *Globba leucantha* var. *bicolor*. Species of Zingiberaceae are defined by peculiar floral morphologies (e.g staminodes and a single functional anther) but are rarely studied for their insect pollinators. Surprisingly, *C. ridleyi* was revealed as the single pollinator of *Globba leucantha*'s showy flowers even though the presence of *Apis* sp., *Trigona* sp., and *Amegilla* sp. were observed in the study site. This small carpenter bee collects both pollen and nectar from the ginger flower. Pollen grains were observed attached to its scopal hairs and hairs on the ventral thorax and ventral abdomen.

The bee contacts the flower's sole stigma only during foraging for pollen, where the stigma contacts pollen accumulated on hairs of the bee's ventral thorax and ventral abdomen. This study represents the first pollination report of a *Ceratina* pollinating a species of Zingiberaceae, and only the second report of any *Ceratina* pollinating a flower with complex morphology.

Keywords

lioceratina, limestone forest, pollinator, wild ginger, xylocopinae

Introduction

Bees are the well-known insect pollinator, especially in global crop pollination (Pattemore 2017). There are about 17,000 bee species in the world (Michener 2007). Bees from the genus *Ceratina* are known as small carpenter bees and are widespread around the world (Warrit et al. 2012). Tropical species of *Ceratina* are little studied, while those of the north temperate zone are mostly studied for their nesting and subsocial behaviors (Michener 1974), not pollination or even their pollen preferences.

Very few studies evaluate the pollination value of *Ceratina* bees. One study reported them to contribute to the overall seed set of *Heterotheca* (Asteraceae) although they were relatively inefficient (Olsen 1996). An Asian species of *Ceratina* was documented to visit and possibly pollinate the complex flowers of an orchid (Orchidaceae) (Li et al. 2008). In general, the species of *Ceratina* are typically broad pollen generalists (polylectic), mostly reported from generalist flowers (e.g. Asteraceae) (Olsen 1996). *Ceratina ridleyi* from the subgenus *Lioceratina* is distributed in Malaya, Sumatra, and Java region (Van der Vecht 1952). This species was described by Cockerell in 1910, and a detailed species description was given by Van der Vecht in 1952. However, the floral-*C. ridleyi* interaction remains unexplored since then.

The family Zingiberaceae (common name: gingers) from the order Zingiberales is largely confined to Southeast Asia and one of the families less studied for plant-pollinator interaction. Ginger plants have hermaphrodite flowers with a single anther and are often bright in color (Larsen et al. 1999). The mutualistic interaction between ginger and pollinator was initially recorded primarily in Asia a few decades ago, which described pollination of several ginger species (Kato et al. 1993; Kittipanangkul and Ngamriabsakul 2006; Aswani et al. 2013; Aswani and Sabu 2018; Rezende et al. 2021). The pollinating activity is dominated by melittophily (pollination by bees), only moderately by cantharophily (beetle) and ornithophily (birds). *Globba* is a genus generally known as dancing lady ginger and unique for its vibrant colored inflorescence. The flower has a single stamen and stigma, the latter extending from the ventral part of the anther. Pollinators of *G. leucantha* var. *bicolor* were not described previously. The record on the pollinating behavior of a small carpenter bee (*Ceratina ridleyi*) presented here is the first documented report on the interaction of the bee with a ginger flower, *G. leucantha* var. *bicolor* in specific.

Methods

Study site

This study was conducted in the limestone forest of Gua Setir (5°39.92'N, 101°55.48'E) at Jeli, Kelantan, Malaysia, in October 2020 and October 2021, as shown in Fig. 1 (QGIS, V3.18.2). Setir cave is in limestone (Liew et al. 2021) and surrounded by small patches of dense secondary forest. The study site is located in a rural area of Jeli district at an elevation of ca 86 m (measured with Global Positioning System, GARMIN GPSMAP 64s, Malaysia).

Study species and phenology observation

Globba leucantha var. *bicolor* is a slender herb, generally distributed in a shady forest, specifically in a damp and humid microhabitat. The plant was identified based on characters described in Holttum (1950) and Larsen et al. (1999). The lifespan and anthesis of a flower were observed in an undisturbed individual plant via a camera trap (RECONYX, U.S.A).

Floral measurements

Morphometry of 38 freshly picked flowers was measured using a digital vernier caliper (MITUTOYO, Japan). Measurement procedures were done in the field between



Figure 1. Location map of the study site, Gua Setir at Jeli, Kelantan, Malaysia. The map was generated via QGIS, V3.18.2.

08:30h to 09:30h. Anther length (AL), anther width (AW), lip length (LL), lip width (LW), flower length (FL), flower width (FW), front opening length (FOW), and lateral opening width (LOW) were measured for each flower (Fig. 2).

Pollinator observations and identification

Three individual plants of *G. leucantha* were observed by two people for three days each to identify the presence of pollinators through the direct observation method (Arumugam et al. 2021). Pollinator visiting times duration for each visit were recorded using a stopwatch. The observation was done from 07:00h to 20:00h. The pollinator was photographed in the field with the aid of a digital camera with an additional portable super macro conversion lens. Samples of pollinators were collected and preserved in 70% ethanol. Identification of pollinator species was done using Van der Vecht (1952) and Warrit et al. (2012) with aid of an image analyzer attached to a stereomicroscope (OPTIKA, Italy).

Pollen analysis

Pollen grains were collected from flowers and hind leg of the pollinator. Collected pollen grains were stained using Safranin (R&M Chemicals, UK) and observed under a compound light microscope attached to an image analyzer (LEICA, Germany). The structure of pollen grains was compared to verify the pollen grains were from the same floral species.

Results

Floral phenology and morphometry

An individual plant of *Globba leucantha* var. *bicolor* forms a single terminal inflorescence that produces one to ten flowers per day for a few months. A matured bud slowly

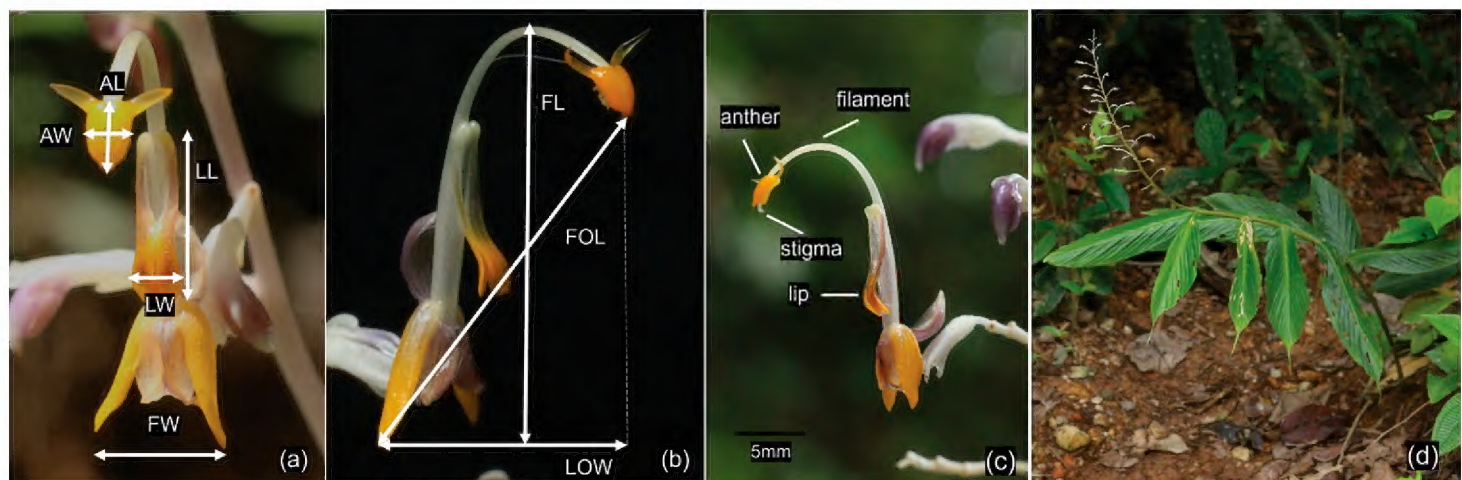


Figure 2. Floral morphometry and morphology of ginger *Globba leucantha* **a** AL: Anther length, AW: Anther width, LL: Lip length, LW: Lip width, FW: Flower width **b** FL: Flower length, FOL: Front opening length, LOW: Lateral opening width **c** morphology of *G. leucantha* in lateral view **d** habit of *G. leucantha*.

opens at 01:00 h and fully bloom around 02:15 h. Anthesis occurs for less than a day (around 02:15 h – 20:00 h). Filament begins to roll around 20:00 h, indicating the beginning of the withering process. The floral completely withered around 23:40 h. Morphometry analysis for 38 flowers is shown in Table 1. The flower length is $21.92 \text{ mm} \pm 1.79$. The anther size is $3.31 \text{ mm} \pm 0.18$ in length and $2.11 \text{ mm} \pm 0.03$ in width. The lip length is $10.65 \text{ mm} \pm 0.19$ and the lip width is $3.18 \text{ mm} \pm 0.23$.

Pollinator morphology

A small carpenter bee, *Ceratina ridleiy* Cockerell, 1910 was identified as the single pollinator of *G. leucantha* var. *bicolor* (Fig. 3). Pollinating behavior was shown by female (♀) *C. ridleiy* only. The body of *C. ridleiy* (♀) is brownish-black with extensive yellow marking (Fig. 4), length ranges from 10.23 mm to 10.28 mm ($n = 2$). The head is enclosed with broad lateral yellow marking including almost entire paraocular areas (Fig. 4E red arrow), four longitudinal yellow lines were noted on the mesonotum (Fig. 4E yellow arrow), and yellow markings were observed in all abdominal tergites (Fig. 4E blue and orange arrow). The hind tibiae consist of scopal hairs. The maximum head length ranges from 2.34 mm to 2.90 mm ($n = 2$), and maximum head width ranges from 3.01 mm to 3.37 mm ($n = 2$).

Pollinator observations and pollen analysis

The small carpenter bee, *C. ridleiy* visited the flowers from 08:00 h to 15:00 h (Fig. 5). The highest foraging activity was recorded in the mid-morning. A total of 103 visits were recorded during nine days of observation of three individual plants, with at least five visits to a plant. Female *C. ridleiy* visited the flower briefly for nectar and pollen collections while the visiting duration was short, ranging only from three sec to 15 sec. Pollen grains collected from both *C. ridleiy* (Fig. 6A) and *G. leucantha* (Fig. 6B) matches in size and structure. The pollen grain is white, spherical and the pollen wall is covered with shorter spicules, diameter ranging from 0.038 mm to 0.045 mm (0.042 ± 0.002 , $n = 12$). Only pollen of *G. leucantha* was found attached to the body of collected *C. ridleiy*.

Table 1. Floral morphometry analysis for ginger *Globba leucantha* from Gua Setir, Malaysia. $\bar{x}$ = mean; SD = standard deviation.

Floral Structures	$\bar{x} \pm \text{SD}$	Range
Anther length, LL (mm)	3.31 ± 0.18	3.16–3.59
Anther width, AW (mm)	2.11 ± 0.03	2.06–2.14
Lip length, LL (mm)	10.65 ± 0.19	10.44–10.93
Lip width, LW (mm)	3.18 ± 0.23	2.96–3.43
Flower length, FL (mm)	21.92 ± 1.79	16.07–25.28
Flower width, FW (mm)	5.46 ± 1.31	2.88–7.70
Front opening length, FOL (mm)	20.62 ± 1.91	15.26–24.27
Lateral opening width, LOW (mm)	13.25 ± 2.47	8.68–19.87

(N = 38 flowers).

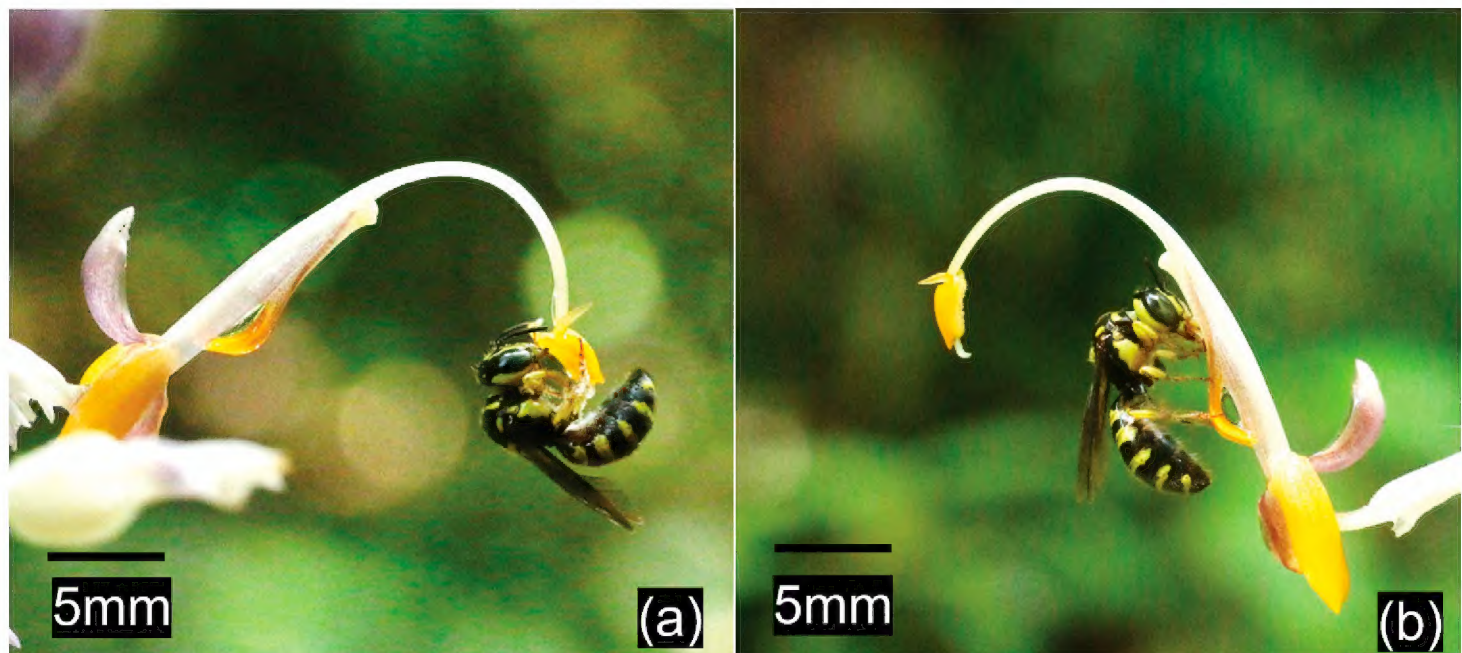


Figure 3. Presence of small carpenter bee, *Ceratina ridleyi* (♀) in ginger flower, *Globba leucantha* **a** bee collecting pollen grains from anther of the ginger flower **b** bee collecting nectar while resting on the lip of ginger flower.

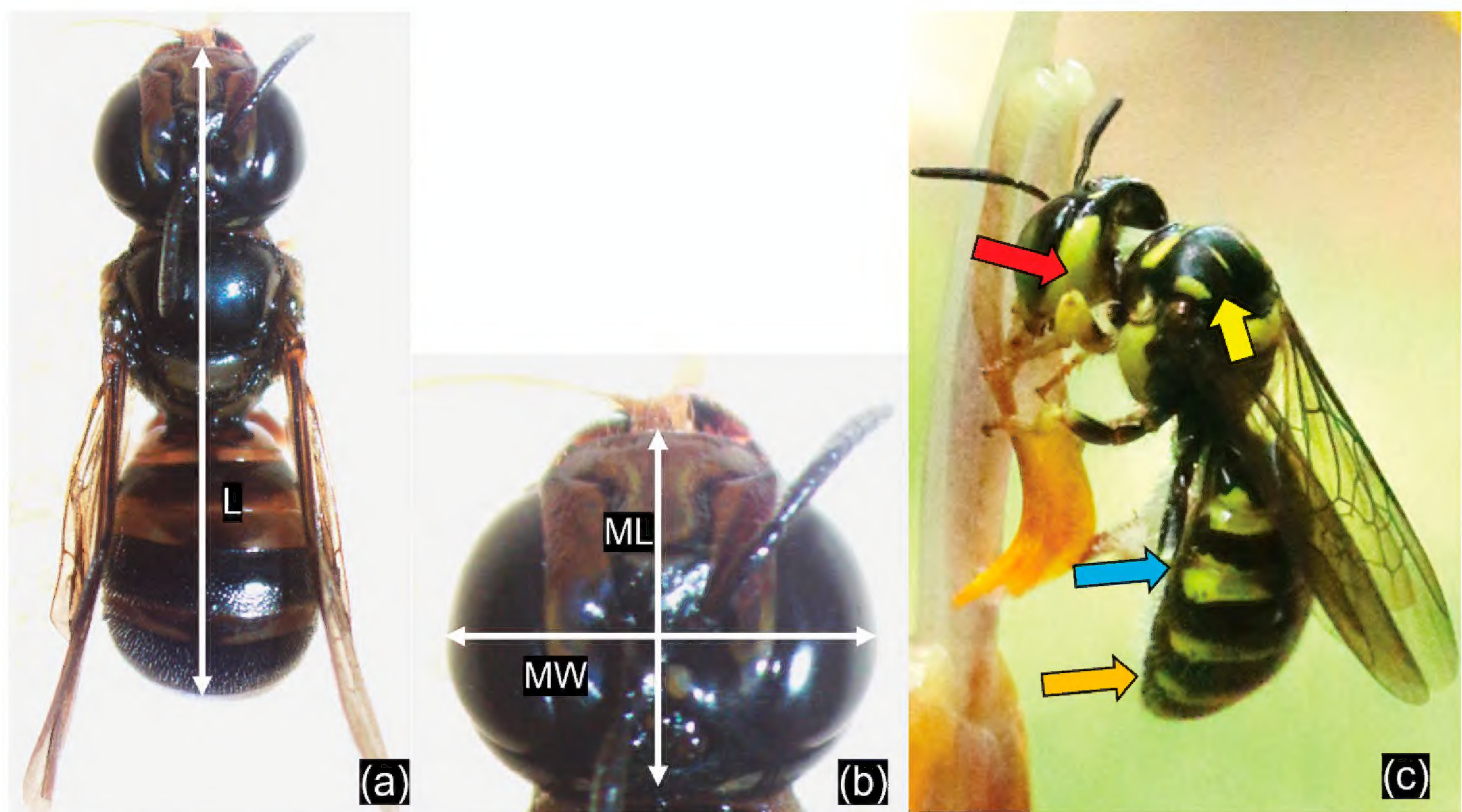


Figure 4. Morphometry and morphology of *Ceratina ridleyi* **a** dorsal view **b** head **c** distinguishable yellow marking on head (red arrow), thorax (yellow arrow) and abdomen (blue and orange arrow). The morphometric measurements are (L) body length, (ML) maximum length, and (MW) maximum width.

Contacts between *C. ridleyi* and *G. leucantha* involves several body parts. Anther and lip were two standard positions on the flower occupied by the bee (Fig. 3); legs hold the anther by head facing ventral part of the anther, body structure curved upward while holding the anther (Fig. 3A); legs settled below the middle part of the lip while inserting the proboscis into the opening of the lip (Fig. 3B). Mouthparts were used to scrape pollen grains from the anther. Pollen grains were observed attached to

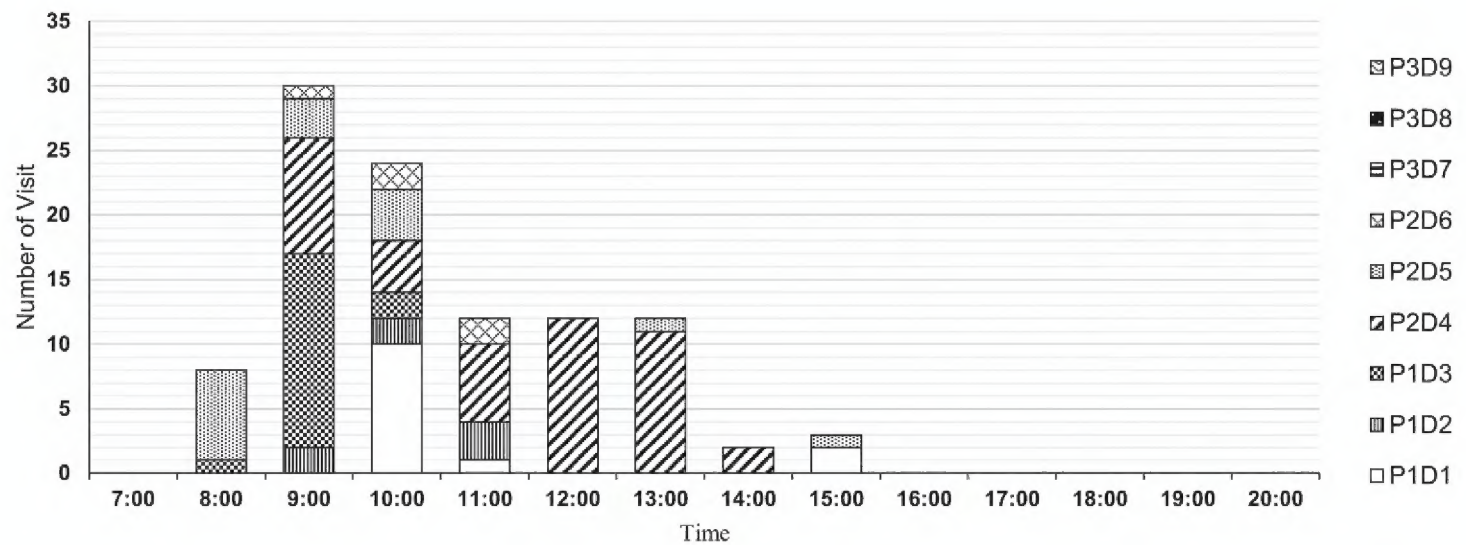


Figure 5. Number of visits to ginger flower, *Globba leucantha* according to time by small carpenter bee, *Ceratina ridleyi*. The graph represents observation data for nine days in Gua Setir, Malaysia. P = plant, D = day.

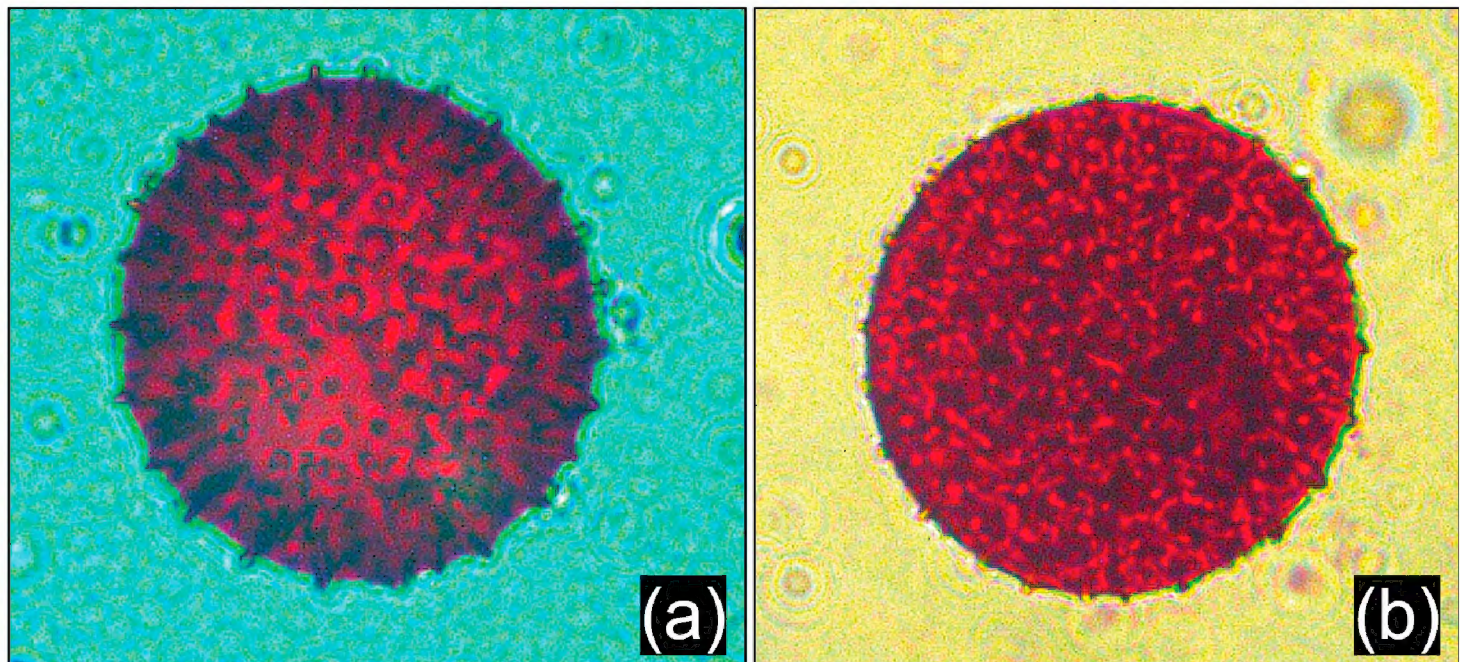


Figure 6. Structure of pollen grain viewed under 40× magnification. The diameter of 12 pollen grains ranges from 0.038mm to 0.045mm **a** a pollen grain of *Globba leucantha* **b** pollen grain collected from the pollinator, *Ceratina ridleyi*.

the hairs in the ventral body parts and legs of the bee during the pollen scrapping activity (Fig. 7). The bee was seen moving from one flower to another in the same inflorescence. It rarely just hovers around the flower without touching any parts of the flower.

Discussion

This study reveals that *Ceratina ridleyi*, a small carpenter bee is the single pollinator of *Globba leucantha* var. *bicolor* in the study area. Blue-banded bees, carpenter bees, halictid bees, honey bees, and stingless bees are among those previously recorded pollinating ginger flowers (Sakai et al. 1999; Zhang et al. 2003; Kittipanangkul and Ngamriabsakul 2006). Small carpenter bees previously were not recorded in Zingiberaceae,

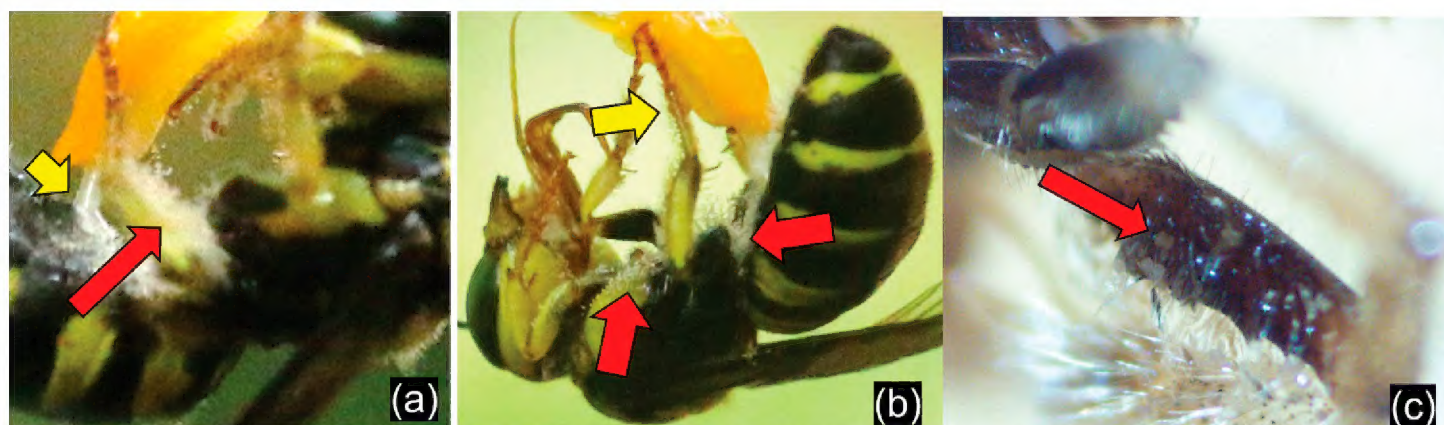


Figure 7. Small carpenter bee, *Ceratina ridleyi* (♀) collecting pollen in ginger flower, *Globba leucantha* **a** scopa in hind leg (red arrow) covered with pollen grains, direct contact of stigma (yellow arrow) and abdomen **b** hairs in the ventral body part (red arrows) and hind leg (yellow arrow) covered with pollen grains **c** observation of pollen grains on hind femur under a stereomicroscope (2 × magnification).

although various solitary bees are known as key pollinators in several ecosystems (Garibaldi et al. 2013; Kline and Joshi 2020). This study, then, is the first record of a small carpenter bee as a pollinator for Zingiberaceae.

Globba leucantha shows a high degree of specificity in pollination, similar in *G. aurantiaca*, Indonesia (Kato et al. 1993), *G. brachyanthera*, Malaysia (Kato 1996; Sakai et al. 1999), and *G. schomburgkii*, India (Aswani and Sabu 2018). Interaction with pollinators was only observed with *C. ridleyi* in *G. leucantha*. Other bees like *Apis* sp. (honey bee), *Trigona* sp. (stingless bee), and *Amegilla* sp. (blue-banded bee) were observed either hovering around the plant or visiting the flower for nectar in the study site.

Open habitats are the general preference of *Ceratina* (Nabhan and Mckibben 2013). In contrast, the presence of *Ceratina* was recorded from a shaded forest area in this study. Despite being considered a generalized pollinator, only pollen grains of *G. leucantha* were observed in the body of *C. ridleyi*. This observation shows that the bees are mon-olectic during the flowering period of *G. leucantha*. Scopal hairs which were identified in *C. ridleyi* during all the 103 floral visits indicated that the females preferred to collect pollen and nectar in *G. leucantha* compared to the males (Fig. 7A). Generally, female bees forage for food and specialized for pollination with the presence of scopal hairs and body hairs in the ventral thorax and ventral abdomen (Tang et al. 2019) as shown in Fig. 7. Pollen collection through body hairs (thorax and abdomen) in *Ceratina* spp. is rarely documented. In future, pollen proportion and pollen carrying strategy in *C. ridleyi* could be studied in detail using the information provided in this study as pioneer data.

Direct contact between *C. ridleyi* and *G. leucantha* results in successful pollination. Brief plant-pollinator interactions were observed, lasting three to 15 sec, mainly involving the collection of pollen (anther) and nectar (lip). The bee primarily touched either anther or lip and was never observed to walk about on the flower. Pollen grains of *G. leucantha* var. *bicolor* are white and medium in size, range within 25–50µm based on pollen grains size category by Kremp (1965). Upon observations, mouthparts of pollinating bees were used to scrape pollen grains while in the hanging position. Sprinkles of pollen were observed during the scrapping process which were then found attached to the scopal hairs, and hairs in

the ventral thorax and ventral abdomen. Direct contact between the ventral abdomen and stigma that extended from anther occurs in the hanging position (Fig. 7A, yellow arrow).

The ginger flower morphology and morphometry facilitates pollinating activity by the small carpenter bee as shown in the Table 1. The flower has a bigger mean front opening length ($20.62\text{mm} \pm 1.91$) and mean lateral opening width ($13.25\text{mm} \pm 2.47$) which give space for the movement of 10mm length sized *C. ridleyi* around the flower especially to land smoothly on the lip and anther. In contrast, the anther is three times smaller than the bee size with a mean length of $3.31\text{mm} \pm 0.18$ and mean width $2.11\text{mm} \pm 0.03$. The head of *C. ridleyi* with 2.62mm mean length and 3.19mm mean width is slightly bigger than the size of the anther. The pollinating bee is able to hold and consume pollen from the anther despite the size difference. The filament and anther of the flower are flexible where they can bend and tolerate the weight of the pollinating bee. This interaction shows that, the bee and flowers are assisting each other for a successful pollination.

Conclusion

Ceratina ridleyi is the specific pollinator of *Globba leucantha* var. *bicolor* at our study site in Malaysia. This is the first report documenting the plant-pollinator interaction between this ginger plant and a bee. We also discuss the floral phenology of *G. leucantha*. Information provided in this report is a pioneer and essential data for future pollination studies of ginger and behavior study of *C. ridleyi*.

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